Immunoresponsiveness in Endometriosis: Implications of Estrogenic Toxicants

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Endometriosis is a reproductive disease characterized by the growth of endometrial cells at sites outside the uterus. This disease is a serious disorder associated with chronic pain and infertility, which may be present in 6 million women in this country. Traditional medical therapy has consisted of hormonal regimens that limit the action of endogenous estrogen. The etiology of endometriosis is unknown, but studies suggest that soluble factors known as cytokines play a role in disease pathogenesis. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) is an environmental toxicant that alters the action of estrogen in reproductive organs and adversely affects immunocompetence. The incidence of endometriosis was determined in rhesus monkeys that were chronically exposed to dioxin for a period of approximately 4 years. Ten years after termination of dioxin treatment, the presence and severity of endometriosis was assessed by surgical laparoscopy. The incidence of endometriosis correlated with dioxin exposure and disease severity was dependent upon the dose administered. Moderate to severe endometriosis was not found in control animals but was documented in three of seven animals exposed to 5 ppt dioxin (43%) and in five of seven animals exposed to 25 ppt dioxin (71%). The frequency of spontaneous disease in the control group was 33%, similar to an overall prevalence of 30% in 304 rhesus monkeys with no history of dioxin exposure. This study indicates that endometriosis may be associated with dioxin exposure in the rhesus. In view of overwhelming evidence that cytokines participate in the mediation of reproductive–endocrine phenomena and regulation of endometrial growth, future assessment of the effects of environmental toxicants on reproductive health may depend upon our understanding of the bidirectional cytokine network between the immune and endocrine systems. — Environ Health Perspect 103(Suppl 7):151–156 (1995)

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Introduction

Endometriosis—The Reproductive Disease

Endometriosis is often described as one of the most enigmatic disorders affecting the reproductive health of women. This disease is defined as the growth of endometrial cells at sites outside the uterus and is characterized by infertility, chronic pain, and adhesion formation. Studies estimate that the prevalence of endometriosis is 10% among reproductive-age women (*1*), indicating that this disease may be present in 6 million women in this country (*2*). The etiology of endometriosis is unknown, and specific factors that contribute to disease progression have not been clearly identified. It is generally accepted that ectopic cells either implant and proliferate following retrograde menstruation or differentiate from a primitive progenitor cell population within the abdominal cavity (*3,4*). It has been demonstrated, however, that retrograde menstrual flow occurs in most reproductive-age women (*5*), yet only a percentage will develop this disease.

Based upon the belief that steroids are the major regulators of the growth and function of ectopic endometriotic tissue, the cornerstone of medical therapy for endometriosis has consisted of modulating the endogenous hormonal environment (*6*). Ectopic endometrial cells can respond to ovarian steroids and undergo cyclic menstrual changes with periodic bleeding. Hormonal regimens available for the treatment of endometriosis include gonadotropin-releasing hormone agonists, the androgen agonist danazol, progestins, and surgical treatments such as oophorectomy. These treatments create an acyclic, low-estrogen endocrine environment, which prevents bleeding, causes atrophy of ectopic implants, and possibly minimizes retrograde reseeding. However, the role of steroids in the growth of endometriotic tissue is unclear. Normal uterine endometrium undergoes predictable histological and biochemical changes in response to hormones throughout the cycle. Studies indicate that the hormonal responsiveness of endometrial implants may be altered. Reports have shown that ectopic

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Abbreviations used: IL, interleukin; PCB, polychlorinated biphenyl; ppt, parts per trillion; rAFS, revised American Fertility Society; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TNF, tumor necrosis factor.

endometrial tissue is histologically asynchronous with corresponding endometrium and may display decreased or variable estrogen and progesterone receptors (*7,8*). Thus, the growth of endometrial implants may be differentially regulated compared to the uterine endometrium.

Immune–Endocrine Regulation of Uterine Endometrial Cell Growth

Human endometrium is a complex tissue composed of glandular structures and endometrial stroma intimately associated with aggregates of lymphoid cells (*9*). It is thought that the biochemical and morphological changes that occur in endometrium during the menstrual cycle are primarily regulated by estrogen and progesterone. However, it is unclear whether the effect of steroid hormones is mediated directly on target cells or exerted indirectly through the elaboration of soluble factors within the endometrium. A series of recent studies indicate that cytokines including interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 are produced by immune and endocrine cell populations within the uterine environment and participate in the growth of endometrium (*10–13*). In addition, these factors may be under the controlling influence of steroid hormones (*12–17*). Thus, interactions between endometrial stromal, epithelial, and lymphoid cells within the uterine environment may be mediated by cytokines in collaboration with steroids.

Immune–Endocrine Interactions via Cytokines

Data also suggest that cytokines including IL-1, TNF, and IL-6 may alter endometrial functions as a result of their direct actions on the hypothalamus, pituitary, or gonads (*10*). Furthermore, cytokines may be important mediators of reproductive-endocrine phenomena via steroid regulation of cytokine secretion in target tissues. Receptors for 17β-estradiol have been demonstrated in peripheral blood leukocytes (*18*), thymic lymphocytes (*18,19*), and lymphoid cells present in human endometrium (*20*). Studies indicate that either *in vitro* or *in vivo* exposure to this steroid or pituitary peptides affects cytokine secretion and gene expression by leukocytes and endometrial cells (*12–17*). For example, peripheral blood leukocytes spontaneously release significantly increased levels of IL-1 and IL-6 after oophorectomy as compared to women who had undergone hysterectomy without removal of ovaries (*16*). Moreover, ovarian ablation was accompanied by alterations in serum levels of plasma proteins associated with bone loss.

Immune Alterations in Endometriosis

Growth factors and inflammatory mediators produced by activated peritoneal leukocytes have been postulated to play a role in the pathogenesis of endometriosis by facilitating the growth of endometrial cells at ectopic sites (*21*). Peritoneal leukocytes are present in increased numbers and exist in a state of heightened activation in patients with this disease relative to normal control women (*21–24*). Peritoneal macrophages from patients with mild endometriosis spontaneously produce IL-1 (*25*), and increased levels of TNF (*26*) and IL-6 (*27*). Elevated levels of inflammatory cell products such as prostaglandins, proteolytic enzymes, complement components, IL-1, and TNF have been observed in the peritoneal fluid of patients with mild endometriosis (*28*). Other work also suggests a pivotal role for peritoneal macrophages in the establishment and maintenance of endometriosis. Isolated populations of macrophages secrete a number of cytokines capable of positively influencing endometrial cell growth, and peritoneal fluid obtained from women with endometriosis increases endometrial stromal cell proliferation (*29–31*). In view of overwhelming evidence for the contribution of cytokines in the regulation of endometrial function, we have postulated that endometriosis is characterized by a dysregulation of leukocyte cytokine production that affects disease progression by influencing the growth of endometrial cells. In this regard, we have shown that peritoneal leukocytes and endometrial cells from patients with endometriosis exhibit altered IL-6 responses that may result in unregulated ectopic endometrial cell growth (*27,32,33*).

Effects of Dioxin on the Reproductive and Immune Systems

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) is a potent chemical toxicant that serves as the reference compound for a large class of halogenated aromatic hydrocarbons (*34*). Extensive evidence using animal model systems demonstrates that both the reproductive and immune systems are targets for dioxin toxicity; however, the effects of this toxin in humans are not clear (*35–40*). Many of the biochemical and toxic effects of dioxin appear to be mediated via binding to an intracellular protein known as the aryl hydrocarbon receptor. Receptor activation follows stereospecific ligand binding, and interaction of the receptor–ligand complex with the DNA-responsive element results in transcriptional activation (*41*). Target genes for the action of dioxin include cytochrome P450 and growth regulatory genes involved in both inflammation and differentiation, including plasminogen activator inhibitor-2 and IL-1β (*41,42*). Dioxin also modulates various hormone receptor systems that play a role in uterine function, including estrogen receptor, progesterone receptor, epidermal growth factor receptor, and prolactin receptor (*43,44*). Moreover, this toxicant alters the action of estrogen in reproductive organs in a manner that is both age dependent and target-organ specific (*44,45*). Importantly, dioxin also adversely affects leukocyte production of cytokines known to participate in the regulation of uterine physiology (*10,46–48*).

Our group had the unique opportunity to study the effect of chronic exposure to dioxin on the frequency and severity of endometriosis in the rhesus monkey. This study was originally undertaken 17 years ago to investigate the long-term reproductive effects of exposure to dioxin in the rhesus. Between 1989 and 1992, three dioxin-treated animals died of severe infiltrating endometriosis. These animals and others in the colony displayed symptoms similar to human disease at the onset of menses, including behavior consistent with pain. In view of these findings, a study was initiated to document endometriosis in this colony of monkeys and to determine whether the severity of disease was correlated with exposure to dioxin (*49*). The prevalence of spontaneous endometriosis in the general population of female rhesus monkeys located at the Harlow Primate Laboratory was also determined.

Methods

Detailed methods for this study have been previously described (*49*). All experimental protocols using rhesus monkeys were performed in accordance with the regulations in the "Guide for Care and Use of Laboratory Animals" and the Animal Welfare Act as amended (7 USC 2131 et Sec.) and were approved by the Animal Review Committee of the University of Wisconsin, Madison.

Study Population

Twenty-four feral female rhesus monkeys (*Macaca mulatta*), 6 to 10 years of age, were obtained in 1977 (Hazelton Research Animals, Reston, VA). The animals were

maintained under climate conditions that simulated their natural breeding season. Monkeys were randomly assigned to three groups of eight animals each. Control animals were not exposed to dioxin, animals in the low-dose group were exposed to 5 parts per trillion (ppt) dioxin, and monkeys in the high-dose group were exposed to 25 ppt dioxin; dioxin was administered in the feed for a period of approximately 4 years, from late 1977 to early 1982. Bioaccumulation of dioxin in selected animals was quantitated from fat biopsy specimens obtained at several time points during and after exposure (*37*).

Animals evaluated for the presence of endometriosis consisted of the 17 live monkeys remaining in this colony in 1992 and the three monkeys that died of extensive endometriosis, from which the disease was documented and staged from autopsy notes. Animals that died before June 1992 of causes other than endometriosis ($n = 4$) were not considered. Although endometriosis was not noted at autopsy, the possibility exists that disease was present but was not grossly apparent. Therefore, these animals were excluded from the present analysis to allow conservative interpretation of these findings. (Inclusion of these animals moderately strengthens the statistical significance of the data in this report.)

Autopsy records from female rhesus monkeys housed at the Harlow Primate Laboratory were reviewed to determine the prevalence of endometriosis in the rhesus general population. This analysis included 304 normal noncastrated females ≥4 years of age with no history of dioxin exposure.

Mixing and Quantitation of Dioxin Diets

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dow Chemical, Midland, MI) was prepared as a stock solution by diluting 19.8 µg dioxin in 1.0 ml benzene. One part stock solution was then diluted with 3550 parts acetone; 200 ml of the resulting solution was mixed with 8 kg of monkey chow (Ralston-Purina Co., St. Louis, MO). Additional normal meal was then added to yield 22.7 kg (50 lb) of chow with a final concentration of 50 ppt dioxin. This premix (5- and 25-lb portions) was added to normal meal (final weight 50 lb) to make the 5-ppt and 25 ppt-diets, respectively. Diets were pelleted by the addition of 2 liters of water and 1 liter of glycerine (which served as binders) per 50-lb bag. Dioxin-free chow was prepared for control monkeys as described above, using benzene, acetone, and glycerine. Dioxin was administered by addition to the daily allotment of 200 g of monkey chow; food records documented that the animals consumed an average of 95% of their daily diet. Dioxin content in the feed was verified by gas chromatograph/mass spectrophotometer analysis of selected samples over the 4-year treatment period, as described by Gross et al. (*50*).

Diagnostic Laparoscopies

In June of 1992, 17 monkeys underwent diagnostic laparoscopy in the facilities of the Harlow Primate Center. Surgeries were carried out in random order and in a blinded fashion, without knowledge of the group assignment of each animal. Surgeries were performed under general anesthesia; a 10-mm diagnostic laparoscope was used to inspect the pelvic organs, the anterior peritoneum, the visible bowel surfaces, the liver edge, and the diaphragm. Clinical findings were recorded and documented by photography at the time of surgery. No postoperative complications occurred in any of the animals; the absence of infection was confirmed by normal complete blood cell counts and blood cultures. The presence and severity of endometriosis was determined according to human criteria using the revised American Fertility Society (rAFS) classification system (*51*). This system of classification standardizes disease severity according to the number, size, and location of endometriotic implants and the presence of adhesions (Table 1). The stage of endometriosis is determined by the total number of points assigned to endometriotic lesions and adhesions. Disease was documented at the time of laparoscopy ($n = 17$) or from autopsy notes ($n = 3$).

Statistical Analysis

The relationship between the degree of endometriosis and the exposure to dioxin was evaluated by monotonic regression analysis of the severity of the disease (rAFS point score) versus the cumulative dose of dioxin (dose × duration). The same results were obtained by analyzing disease relative to daily dioxin dose. The Cochran-Armitage trend test was used to evaluate differences in the frequency of endometriosis between control, 5 ppt, and 25 ppt animals. Further evidence that the frequency of disease was increased in dioxin-exposed animals relative to unexposed animals was calculated by utilizing the retrospective data; significant differences in the frequency of disease between groups were determined using the Fisher's exact test.

Table 1. The American Fertility Society Revised Classification System.[a]

Site	Endometriosis	Lesion < 1 cm, points	Lesion = 1–3 cm, points	Lesion > 3 cm, points
Peritoneum	Superficial	1	2	4
	Deep	2	4	6
Ovary				
Right	Superficial	1	2	4
	Deep	4	16	20
Left	Superficial	1	2	4
	Deep	4	16	20
Posterior culdesac obliteration	Partial obliteration 4 points Complete obliteration 40 points			

Site	Adhesion type	Site < 1/3 enclosed, points	Site = 1/3–2/3 enclosed, points	Site > 2/3 enclosed, points
Ovary				
Right	Filmy	1	2	4
	Dense	4	8	16
Left	Filmy	1	2	4
	Dense	4	8	16
Tube				
Right	Filmy	1	2	4
	Dense	4	8	16
Left	Filmy	1	2	4
	Dense	4	8	16

[a]Stage of endometriosis is determined by the total number of points assigned to endometriotic lesions and adhesions. Stage I (Minimal) 1–5 points; Stage II (Mild) 6–15 points; Stage III (Moderate) 16–40 points; Stage IV (Severe) > 40 points.

Results

The presence and severity of endometriosis in this colony of 20 rhesus monkeys are shown in Table 2. By rAFS staging, control animals not exposed to dioxin exhibited either no endometriosis (4 of 6 animals) or had minimal disease present (2 of 6 animals). In contrast, disease was absent in 2 of 7 monkeys that received 5 ppt dioxin, whereas only 1 of the 7 animals exposed to 25 ppt dioxin was disease free. Moderate to severe disease was not seen in control animals but was documented in 3 of 7 (43%) animals exposed to 5 ppt toxicant. In monkeys exposed to 25 ppt dioxin, moderate to severe endometriosis was present in 5 of 7 (71%) animals. Statistical analysis revealed that the severity of endometriosis was significantly correlated with the dose of dioxin administered (Figure 1).

The prevalence of spontaneous endometriosis among rhesus monkeys housed at the Harlow Primate Laboratory was examined retrospectively by analysis of pathology notes recorded at the time of routine autopsy. This analysis included 304 normal noncastrated females of reproductive age (≥4 years of age) with no history of dioxin exposure. Of animals surveyed, 135 were between 4 and 13 years of age, while 169 monkeys were 13 years of age or more. Endometriosis was not seen in animals less than 13 years old. In contrast, disease was documented in 51 of 169 (30%) animals ≥13 years of age. The prevalence of spontaneous disease in animals of 13 years of age or greater is in agreement with that seen for the control animals evaluated in the present study (2 of 6 controls, 33% frequency). The data presented in Table 3 demonstrate that the frequency of endometriosis was also increased in dioxin-exposed animals as compared to animals with no history of dioxin exposure.

Discussion

The results of these studies demonstrate that chronic exposure to the chemical toxicant dioxin is directly correlated with the increased presence and severity of endometriosis in rhesus monkeys. As determined by the rAFS scoring system, Stage II, III, and IV disease was exclusively found in animals exposed to either 5 ppt or 25 ppt dioxin. Furthermore, the severity of disease, as reflected by the rAFS point score, was positively correlated with the daily and cumulative dose of dioxin administered.

Despite intense research efforts over the past 40 years, the pathophysiology of endometriosis remains incompletely understood. Research has been hindered because this disease occurs exclusively in menstruating species, including humans and nonhuman primates. The rhesus monkey is a suitable model because endometriosis develops spontaneously in these animals and resembles human disease anatomically and clinically (*52–54*). Disease manifestations include intraabdominal cyst formation and adhesions involving the ovaries, ureters, colon, or urinary bladder. As noted in our study, other investigators have described that animals with endometriosis exhibit behavior consistent with pain, including prostration and anorexia occurring with the menses (*54–57*). A unique feature of endometriosis in the rhesus monkey, unlike human disease, is the potential for disease fatality, particularly if intestinal blockage occurs. Unfortunately, endometriosis is frequently unrecognized in monkeys until late stages of disease, and euthanasia for humane reasons may be carried out due to severe pain (*54,57*). However, reports indicate that rhesus disease may respond to surgical techniques or hormonal therapy as employed to treat human endometriosis (*57,58*).

The prevalence of spontaneous endometriosis in animals with no history of dioxin exposure found in our work is similar to the frequency of 26% reported in a controlled study by Fanton and Golden (*54*). The absence of disease recorded at autopsy in animals less than 13 years of age suggests that the prevalence of clinically detectable endometriosis may increase with advancing age, as found in human and rhesus studies (*54,59*). Moreover, disease may not be visually apparent at autopsy in animals before 13 years of age, possibly due to age related changes in the appearance of lesions as noted in humans (*59*). The prevalence of spontaneous endometriosis in the rhesus and the association of advancing age with an increased frequency of endometriosis is an intriguing finding and is under further investigation.

An association of endometriosis in rhesus monkeys following exposure to polychlorobiphenyl (PCB) compounds has been previously described (Campbell et al., unpublished results). Our study supports and extends these findings, since dioxin is used a reference compound for halogenated aromatic hydrocarbons, including PCBs (*34*). An increased incidence of endometriosis in the rhesus has been documented following exposure to single-energy proton irradiation, mixed-energy proton irradiation, and X-rays (*54,60,61*). The shortest time lapsed between irradiation exposure and the development of endometriosis in these studies was 6 years (*61*). In our study, endometriosis in dioxin-exposed monkeys was first documented 7 years following the termination of dioxin treatment. Immune system defects are a common probable factor that may contribute to the development of endometriosis in each of these animal models. Indeed, this notion is consistent with human studies, suggesting that immune mechanisms may contribute to the disease process (*62,63*).

Recent reports suggest that acute dioxin exposure in rodents alters leukocyte production of the inflammatory mediators

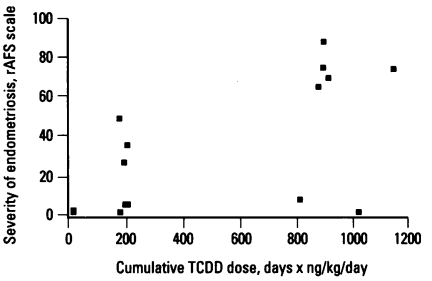

Figure 1. The severity of endometriosis in rhesus monkeys versus the cumulative dose of dioxin. Monotonic regression analysis revealed that the severity of endometriosis (rAFS point score) was significantly correlated with the dose of dioxin administered. $p<0.001$, 5 and 25 ppt animals compared with corresponding values for controls; $p<0.01$, 5 ppt animals compared with corresponding value for controls; $p<0.05$, 25 ppt animals compared with corresponding value for 5 ppt animals.

Table 2. Severity of endometriosis in dioxin-treated rhesus monkeys.[a]

	Classification				
Group	None	I	II	III	IV
Control	4	2	0	0	0
5 ppt	2	2	0	2	1
25 ppt	1	0	1	1	4

[a]Stage of disease was determined according to the revised American Fertility Society classification system. Disease was evaluated at the time of diagnostic laparoscopy ($n=17$) or from autopsy notes ($n=3$).

Table 3. Frequency of endometriosis in dioxin-exposed rhesus monkeys.

Groups	Statistical test	p Value[b]
All groups ($n=20$)	Cochran-Armitage	$p<0.05$
Controls[a] (51/169) versus 5 ppt (5/7)	Fisher's Exact	$p=0.034$
Controls[a] (51/169) versus 25 ppt (6/7)	Fisher's Exact	$p=0.005$

[a]Controls with disease (30.2%) were female animals ≥13 years of age housed at The Harlow Primate Center with no history of dioxin exposure. [b]All p values are one-tailed.

TNF and IL-6, which may play a role in dioxin toxicity (*46–48,64*). In addition, previous studies in our laboratory (*27*) and others (*26*) have shown that peritoneal leukocyte populations from patients with endometriosis exhibit aberrant patterns of IL-6 and TNF secretion. Since circulating leukocytes represent the pool for repopulation of these cells within the peritoneal cavity, additional work is in progress to examine the ability of peripheral blood mononuclear cells obtained from these rhesus monkeys to produce TNFα and IL-6. Preliminary data from these studies indicate that endometriosis or toxicant exposure was associated with altered cytokine production in response to endotoxin stimulation by rhesus peripheral blood leukocytes *in vitro* (*65*). These experiments provide evidence that suggests the possibility of a dysregulation of cytokine production peripherally in monkeys exposed to dioxin or animals with endometriosis and lend further support to the hypothesis that this disease may be characterized with systemic immune alterations.

The etiology of endometriosis remains elusive, and the potential role of dioxin in the pathogenesis of this disease is unclear. However, the study of cytokine production by peritoneal and peripheral leukocytes and endometrial cells and the effect of these cytokines on endometrial cell growth may provide new directions for research. In addition, the effect of dioxin on the growth of uterine and ectopic endometrial cells and cytokine–steroid interactions is not known and awaits future investigation. Dioxin exerts known effects on the immune system, including stimulation of the secretion of cytokines, which participate in the regulation of endometrial function (*10,11,46–48*). In addition, this toxicant alters tissue-specific responses to hormones via modulation of steroid receptor expression (*44*). Chronic unregulated cytokine secretion by leukocytes and endometrial cells in combination with hormonal dysregulation may have facilitated the aberrant growth of endometrial tissue within the peritoneum of dioxin-treated animals. The serendipitous finding of endometriosis in rhesus monkeys exposed to dioxin highlights the need for collaboration between clinicians and researchers within the fields of gynecology, immunology, neuroendocrinology, and toxicology to gain more insight into the effects of environmental toxicants on human reproductive health.

REFERENCES

1. U.S. Bureau of Census. Statistical Abstracts of the United States. Washington:U.S. Bureau of Census, 1990;14.
2. Wheeler JM. Epidemiology and prevalence of endometriosis. Infertil Reprod Med Clinics NA 3:545–549 (1992).
3. Sampson JA. The development of the implantation theory for the origin of peritoneal endometriosis. Am J Obstet Gynecol 14:549–557 (1940).
4. Schweppe K-W. Endometriotic lesions: location, gross, histologic and structural aspects. In: Current Concepts in Endometriosis, Progress in Clinical and Biological Research. Vol 323 (Chadha DR, Buttram VC Jr, eds). New York:Alan Liss, 1990;33–47.
5. Koninckx PR, Ide P, Vandenbrouck W, Brosens IA. New aspects of the pathophysiology of endometriosis and associated infertility. J Reprod Med 24:257–260 (1980).
6. Olive DR, Schwartz LB. Endometriosis. N Engl J Med 328:1759–1769 (1993).
7. Metzger DA, Olive DL, Haney AF. Limited hormonal responsiveness of ectopic endometrium: histologic correlation with intrauterine endometrium. Hum Pathol 19:1417–1424 (1988).
8. Lessey BA, Metzger DA, Haney AF, McCarty KS. Immunohistochemical analysis of estrogen and progesterone receptors in endometriosis: comparison with normal endometrium during the menstrual cycle and the effect of medical therapy. Fertil Steril 51:409–415 (1989).
9. Tabibzedeh S. Proliferative activity of lymphoid cells in human endometrium throughout the menstrual cycle. J Clin Endocrinol Metab 70:437–443 (1990).
10. Tabibzedeh S. Human endometrium: an active site of cytokine production and action. Endocr Rev 12:272–290 (1991).
11. Tabibzedeh S, Sun XS. Cytokine expression in human endometrium throughout the menstrual cycle. Hum Reprod 7:1214–1221 (1992).
12. Tabibzadeh S, Santhanam U, Sehgal PB, May LT. Cytokine-induced production of IFN-β2 by freshly explanted human endometrial stromal cells. J Immunol 142:3134–3139 (1989).
13. Laird SM, Li TC, Bolton AE. The production of placental protein 14 and interleukin 6 by human endometrial cells in culture. Hum Reprod 8:793–798 (1993).
14. Polan ML, Loukides J, Nelson P, Carding S, Diamond M, Walsh A, Bottomly L. Progesterone and estradiol modulate interleukin-1β messenger ribonucleic acid levels in cultured human peripheral monocytes. J Clin Endocrinol Metab 89:1200–1206 (1989).
15. Ralston SH, Russell RGG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cell in postmenopausal women. J Bone Miner Res 5:983–988 (1990).
16. Pioli G, Basini G, Pedrazzoni M, Musetti G, Ulietti V, Bresciani D, Villa P, Bacchi A, Hughes D, Russell G. Spontaneous release of interleukin-1 and interleukin-6 by peripheral blood mononuclear cells after oophorectomy. Clin Sci 83:503–507 (1992).
17. Jacobs AL, Sehgal PB, Julian J, Carson DD. Secretion and hormonal regulation of interleukin-6 production by mouse uterine stromal and polarized epithelial cells cultured *in vitro*. Endocrinology 131:1037–1045 (1992).
18. Weusten JJ, Blankenstein MA, Gmelig-Meyling FHJ, Schuurman HJ, Kater L, Thijssen JHH. Presence of oestrogen receptors in human blood mononuclear cells and thymocytes. Acta Endocrinol 112:409–414 (1986).
19. Danel L, Sovweine G, Monier JC, Saez S. Specific estrogen binding sites in lymphoid cells and thymic cells. J Steroid Biochem 18:559-563 (1983).
20. Tabibzadeh SS, Satyaswaroop PG. Sex steroid receptors in lymphoid cells of human endometrium. Am J Clin Pathol 91:656–663 (1989).
21. Halme J, Becker S, Haskill S. Altered maturation and function of peritoneal macrophages: possible role in the pathogenesis of endometriosis. Am J Obstet Gynecol 156:783–789 (1987).
22. Haney AF, Muscato JJ, Weinberg JB. Peritoneal fluid cell populations in infertility patients. Fertil Steril 35:696–698 (1981).
23. Halme J, Becker S, Hammond MG, Raj MHG, Raj S. Increased activation of pelvic macrophages in infertile women with mild endometriosis. Am J Obstet Gynecol 145:333–337 (1983).
24. Hill JA, Faris HMP, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 50:216–222 (1988).
25. Fakih H, Baggett B, Holtz G, Tsang K-Y, Lee JC, Williamson HO. Interleukin-1: a possible role in the infertility associated with endometriosis. Fertil Steril 47:213–217 (1987).
26. Halme J. Release of tumor necrosis factor-α by human peritoneal macrophages *in vivo* and *in vitro*. Am J Obstet Gynecol

161:1718–1725 (1989).
27. Rier SE, Parsons AK, Becker JL. Altered interleukin-6 production by peritoneal leukocytes from patients with endometriosis. Fertil Steril 61:294–299 (1994).
28. Ramey JW, Archer DF. Peritoneal fluid: its relevance to the development of endometriosis. Fertil Steril 60:1–17 (1993).
29. Halme J, White C, Kauma S, Estes J, Haskill S. Peritoneal macrophages from patients with endometriosis release growth factor activity *in vitro*. J Clin Endocrinol Metabol 66:1044–1049 (1988).
30. Olive DL, Montaya I, Riehl RM, Schenken RS. Macrophage-conditioned media enhance endometrial stromal cell proliferation *in vitro*. Am J Obstet Gynecol 164:953–958 (1991).
31. Surrey ES, Halme J. Effect of peritoneal fluid from endometriosis patients on endometrial stromal cell proliferation *in vitro*. Obstet Gynecol 76:792–797 (1990).
32. Zarmakoupis PN, Rier SE, Becker JL. Inhibition of endometrial stromal cell proliferation by interleukin-6. Hum Reprod (in press).
33. Rier SE, Zarmakoupis PN, Hsu X, Becker JL. Dysregulation of interleukin-6 responses in ectopic endometrial stromal cells: correlation with decreased soluble receptor levels in peritoneal fluid of women with endometriosis. J Clin Endocrinol Metab 80:1431–1437 (1995).
34. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 22:517–554 (1982).
35. Allen JR, Barsotti LK, Lambrecht LK, Van Miller JP. Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann NY Acad Sci 320:419–425 (1979).
36. McNaulty WP. Fetotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for rhesus macaques (*Macaca mulatta*). Am J Primatol 6:41–47 (1984).
37. Bowman RE, Schantz SL, Weerasinghe NCA, Gross M, Barsotti D. Chronic dietary intake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at 5 or 25 parts per trillion in the monkey: TCDD kinetics and dose-effect estimates of reproductive toxicity. Chemosphere 18:243–252 (1989).
38. Holsapple MP, Snyder NK, Wood SC, Morris DL. A review of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: 1991 update. Toxicology 69:219–255 (1991).
39. Neubert R, Jacob-Muller U, Stahlmann R, Helge H, Neubert D. Polyhalogenated dibenzo-*p*-dioxins and dibenzofurans and the immune system. Arch Toxicol 65:213–219 (1991).
40. Tomar RS, Kerkvliet NI. Reduced T-helper cell function in mice exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol Lett 57:55–64 (1991).
41. Whitlock JP. Genetic and molecular aspects of 2,3,7,8-tetrachorodibenzo-*p*-dioxin action. Annu Rev Pharmacol 30:251–277 (1990).
42. Sutter TR, Guzman K, Dold KM, Greenlee WF. Targets for dioxin: genes for plasminogen activator inhibitor-2 and interleukin-1β. Science 254:415–419 (1991).
43. Jones MK, Weinsenburger WP, Sipes IG, Russell DH. Circadian alterations in prolactin, corticosterone, and thyroid hormone levels and down-regulation of prolactin receptor activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Appl Pharmacol 87:337–352 (1987).
44. Safe S, Astroff B, Harris M, Zacharewski T, Dickerson R, Romkes M, Biegal L. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds as antiestrogens: characterization and mechanism of action. Pharmacol Toxicol 69:400-409 (1991).
45. DeVito MJ, Thomas T, Martin E, Umbreit TH, Gallo MA. Antiestrogenic action of 2,3,7,8-tetrachlordibenzo-*p*-dioxin: tissue-specific regulation of estrogen receptor in CD1 mice. Toxicol Appl Pharmacol 113:284–292 (1992).
46. Taylor MJ, Clark GC, Atkins ZZ, Lucier G, Luster MI. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin increases the release of tumor necrosis factor-alpha (TNFα) and induces ethoxyresurofin-*O*-deethylase (EROD) activity in rat Kupffer's cells (KCs). Toxicologist 10:276–282 (1990).
47. Clark GC, Taylor MJ, Tritscher AM, Lucier GW. Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzp-*p*-dioxin-mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. Toxicol Appl Pharmacol 111:422–431 (1991).
48. Hoglen N, Swim A, Robertson L, Shedlofsky S. Effects of xenobiotics on serum tumor necrosis factor (TNF) and interleukin-6 (IL-6) release after LPS in rats. Toxicologist 12:290–297 (1992).
49. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) chronically exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundam Appl Toxicol 21:433-441 (1993).
50. Gross ML, Sun T, Lyon PA, Wojinski SF, Hilker DR, Dupuy AE, Heath JG. Method validation for the determination of tetrachlorodibenzodioxin at the low parts-per-trillion level. Anal Chem 53:1902–1906 (1981).
51. The American Fertility Society. Revised American Fertility Society classification of endometriosis. Fertil Steril 43:351–352 (1985).
52. McClure HM. Endometriosis. In: Spontaneous Animal Models of Human Disease. Vol 1 (Andrews EJ, Ward BC, Altman NH, eds). New York:Academic Press, 1979;215–218.
53. MacKenzie WF, Casey HW. Animal model of human disease: endometriosis in rhesus monkeys. Am J Pathol 80:341–344 (1975).
54. Fanton JW, Golden JG. Radiation-induced endometriosis in *Macaca mulatta*. Radiat Res 126:141–146 (1991).
55. Lindberg BS, Busch C. Endometriosis in rhesus monkeys. Upsala J Med Sci 89:129–134 (1984).
56. Fanton JW, Hubbard GB, Wood DH. Endometriosis: clinical and pathological findings in 70 rhesus monkeys. Am J Vet Res 47:1537–1541 (1986).
57. Fanton JW, Yochmowitz MG, Wood DH, Salmon YL. Surgical treatment of endometriosis in 50 rhesus monkeys. Am J Vet Res 47:1602–1604 (1986).
58. Mann DR, Collins DC, Smith MM, Kessler MJ, Gould KG. Treatment of endometriosis in monkeys: effectiveness of continuous infusion of a gonadotropin-releasing hormone agonist compared to treatment with a progestational steroid. J Clin Endocrinol Metab 63:1277–1283 (1986).
59. Martin DC, ed. Laparoscopic Appearance of Endometriosis, Color Atlas. 2d ed. Memphis:The Resurge Press, 1991.
60. Wood DH, Yochmowitz MG, Salmon YL, Eason RI, Boster RA. Proton irradiation and endometriosis. Aviat Space Environ Med 54:718–724 (1983).
61. Wood DH. Long-term mortality and cancer risk in irradiated rhesus monkeys. Radiat Res 126:132–140 (1991).
62. Dmowski WP, Braun D, Gebel H. The immune system in endometriosis. In: Modern Approaches to Endometriosis (Thomas EJ, Rock JA, eds). Boston:Kluwer Academic Publishers, 1991;97–111.
63. Hill JA. Immunologic factors in endometriosis and endometriosis-associated reproductive failure. Infertil Reprod Med Clinics of NA 3:583–596 (1992).
64. Taylor MJ, Lucier GW, Mahler JF, Thompson M, Lockhart AC, Clark GC. Inhibition of acute TCDD toxicity by treatment with anti-tumor necrosis factor antibody or dexamethazone. Toxicol Appl Pharmacol 117:126–132 (1992).
65. Rier SE, Spangelo BL, Martin DC, Bowman RE, Becker JL. Tumor necrosis factor alpha and interleukin-6 production by peripheral blood mononuclear cells from rhesus monkeys with endometriosis. J Immunol 150:49A (1993).